Use of artificial refuges by the northern viper *Vipera berus* - 3. An experimental improvement to the thermal properties of refuges

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ABSTRACT - To increase the use of galvanised corrugated-iron refuges ('tins') by northern vipers (*Vipera berus*) in a chalk downland reserve, insulation mats were placed on half of the bare ground area below the tins. During the day time the operative temperatures (T_e) on the mats, demonstrated by physical models, averaged 2-4˚C warmer than bare ground under the same refuges but ranged as much as 10˚C warmer. Vipers were found more frequently under insulated than uninsulated tins and, when under these tins, were more likely to be on the mat than on the bare ground. Adult males on mats in March/early April had body temperatures (T_b) 9.4˚-15.8˚C higher than T_e (which at this time of year is particularly low). At other times adult males, females and juveniles were on average about 1 to 2˚C warmer but, surprisingly, sub-adults showed no temperature difference. Insulation mats, by enabling the vipers to maintain higher T_b, should improve their physiological performance and if they reduce open basking and the incentive to search for warmer positions then they could reduce exposure to avian predators. The inclusion of an insulation mat as a component of an improved refuge design is now under investigation.

INTRODUCTION

Galvanised corrugated-iron refuges ('tins') are frequently used for monitoring reptiles in the UK and elsewhere (Reading 1997; Froglife 1999) and their use by the northern vipers (*Vipera berus*) has been described recently (Hodges & Seabrook, 2016a). They have the advantage of providing locations where there is both warmth and cover, perhaps helping vipers remain concealed and closer to their upper thermal set point ($T_{set\ upper}$), estimated as 32.9˚C (Hodges & Seabrook, 2016b). However, previous study has demonstrated some shortcomings in the thermal performance of these refuges (Hodges & Seabrook, 2016b). In particular, the bodies of vipers beneath tins warm much more slowly than in direct sunshine and that early in the reptile active season the low operative temperatures (T_e) below tin refuges would discourage their use. These disadvantages are expected to reduce viper observation rates at refuges and consequently the value of refuges for monitoring. Their value in conservation may also be reduced since the animals may be more inclined to leave refuge cover periodically to bask in the open to achieve preferred body temperatures and so increase the risk of predation.

In an effort to improve the thermal performance of tin refuges for *V. berus*, we undertook an investigation of the potential benefits of placing an insulation mat on the ground below tins in a 7-month study in a chalk downland reserve.

MATERIALS AND METHODS

Study site and refuges

Details of the study site and refuges are presented in Hodges & Seabrook (2016a). In brief, the investigation was part of a long-term monitoring programme at a chalk downland nature reserve at about 51˚N, 0˚E, with a total open area of 11.1ha. Refuges consisted of galvanised corrugated-iron sheets ('tins') (0.5mm thick and 0.5g/cm2) cut to 50cm by 65cm, and were camouflaged by spraying their upper surface with brown paint (Espresso, satin finish, Rust-oleum). These were placed in sunny but inconspicuous locations backed by vegetation cover. Observations of vipers were made under 46 tins, deployed at a density of about 4/ha; the ground below refuges lacked vegetation.

The study was undertaken in 2015 when the site was visited 77 times for many hours from March to October. Observations were made morning and afternoon on days when weather conditions were not excessively wet or windy. A standard route was followed between refuge locations. Photographs were taken of viper head-scale patterns; these were coded then entered into a database to facilitate individual recognition (Benson, 1999). Adult recruitment tables constructed for the two sites suggested that there were about 3-4 adult vipers/ha in 2015. Life stages were defined as before (Hodges & Seabrook, 2016a) and observations disaggregated by gender except for juveniles. The study involved no animal handling in order to minimise disturbance and stress.

Physical models and temperature measurement

The use of physical models (Fig. 1) to determine operative temperatures (T_e) and infra-red thermometers (Foxnovo DT8380) to measure viper body surface and model temperatures were the same as described in Hodges & Seabrook (2016b). Where possible, temperature measurements were taken from vipers both under and away from tins. The physical models consisted of copper pipe

(ID 20mm, wall 1mm thick, length 150mm) flattened so that about 40% of surface was in contact with the substrate beneath, sprayed with grey paint (Surface primer, matt, Rust-oleum), sealed at either ends with silicon sealant and fixed into the ground with two overlapping strands of wire. A total of 22 tins had models on the ground beneath them and seven also had models on the insulation mat. These seven tins also had two other closely located models, one exposed to direct sunlight and one in the permanent shade of taller vegetation. The temperatures of all models were recorded routinely using an infra-red thermometer at least five times a month in the period 09.00h to 13.00h. Additionally, temperature measurements of vipers under tins also included measurement of any corresponding models. All times given for temperature measurement are Greenwich Mean Time (GMT).

Figure 1. Adult male *V. berus*, below a tin refuge (removed) and resting on an insulation mat. Photograph taken just after body temperature measured; on the left hand side is a physical model for determination of operational temperature.

Temperature loggers (Gemini TK-4014, TinyTag Talk 2, accuracy ±0.4˚C) were used to compare temperatures under tins (on bare ground and on the insulation mat) and elsewhere in direct sunlight and in shade of vegetation. They were used over the course of a day and set to read at 10 minute intervals. The loggers were placed in aluminium cylinders (10cm by 4cm), painted with the same grey undercoat as the physical models, and flattened on one side to increase contact with the ground. The cylinders were held in place using metal skewers and under the tin positioned beneath one of the convex corrugations but not touching the tin.

Below tin insulation

Insulation mats (Fig. 1) measured 30cm x 50cm and consequently covered one half of the ground beneath a tin, the other half was left as bare ground. The mats were prepared from two layers of bubble foil plastic insulation (Baird double aluminium bubble foil insulation 200g/m2 and RSI = 2.93) held together by parcel tape and sprayed above with brown paint for camouflage (Espresso, satin finish, Rust-oleum). The insulation mats were placed alternately under 23 refuges (referred to as insulated tins), leaving 23 without mats (referred to as uninsulated tins). In this way two different comparisons can be made. First, the numbers of vipers recorded under insulated tins can be compared with those under uninsulated tins (are refuge positions with mats favoured over those without?). Second, for insulated tins the numbers of vipers resting on mats can be compared with those resting on the bare ground (are mats preferred to the ground?).

Statistical analysis

Differences between numbers of vipers using insulated and uninsulated tins and using or not using mats under insulated tins were tested for statistical significance by the Mann Whitney U test and the heterogeneity of body temperature measures of the different viper life stages was examined using Kruskal-Wallis one way analysis of variance (Siegal, 1956). The statistical significance of simple linear correlation coefficients (r) was determined from standard tables (Bailey, 1966). Differences between the numbers of viper life stages on mats and the on bare ground under insulated tins were evaluated for statistical significance using χ^2 tests (Siegal, 1956). Differences were treated as statistically significant when the probability of them occurring by chance was 5% or less (p≤0.05).

RESULTS

Observations on the T_e of insulated tins

During each month of the study, temperature measurements from 09.00h to 13.00h showed that models on insulation mats were on average always warmer than those on bare ground (Fig. 2). Models on mats were on average 4.3˚C warmer; their greatest mean temperature advantage was in June (5.7˚C) and least in October (3.3˚C). It was noticeable that in March there was little difference in the temperature between models on bare ground under tins or in the shade of vegetation but thereafter the models on the bare ground became distinctly warmer (Fig. 2).

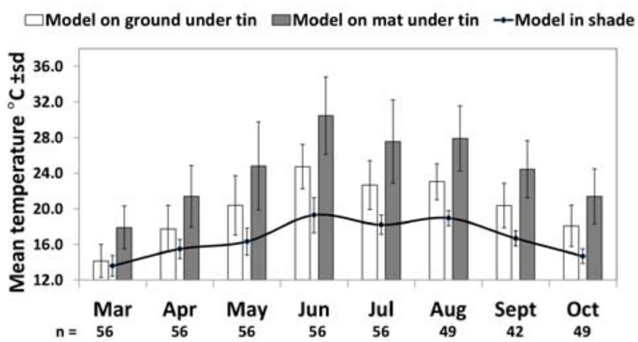

Figure 2. Mean (±sd) monthly temperatures of physical models at seven locations to show differences between operational temperatures under a tin on bare ground or on an insulation mat, or in the shade of vegetation, in the period March to October 2015 (n = number of observations per bar)

As the temperatures of tins increased so the temperature difference between models on the mats and adjacent bare ground became greater (Fig. 3); there was a significant positive correlation between the temperature of the tins and the magnitude of this difference ($r = 0.64$, $df = 54$, $p<0.001$). This relationship was to be expected since rapid rises in temperature of the tin would be expected to have more impact on the model on the mat, which is insulated from the cooling effect of the ground, than on the model on the bare ground.

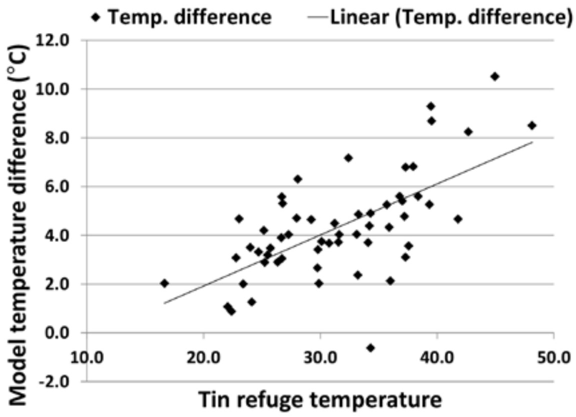

Figure 3. Difference in temperature between the physical models on the mats or adjacent bare ground plotted against the corresponding temperature of tin refuge they were beneath. Assessed 09.00h to 13.00h from March to October (N=56)

For each of the 8 months of the study (March to October), in the period 05.00h to 18.00h the mean temperature differences between mats and the bare ground under the same tins, recorded by loggers, were -0.5°C to just above +4°C (Fig. 4) and from 08.00h to 16.00h were 2°C to 4°C. There were also wide ranging maximum and minimum values (Fig. 4). Negative values, when the insulation mat was actually cooler than the bare ground, occurred from about 17.00h to 09.00h. This appears to result from sharp night time falls in air temperature. In this situation loggers on mats were not buffered by the warmer ground below and so registered lower temperatures.

One possible disadvantage of the insulation mats is that they could heat to temperatures above upper thermal set point ($T_{set\ upper}$) of the vipers (in this case taken as 32.9°C),

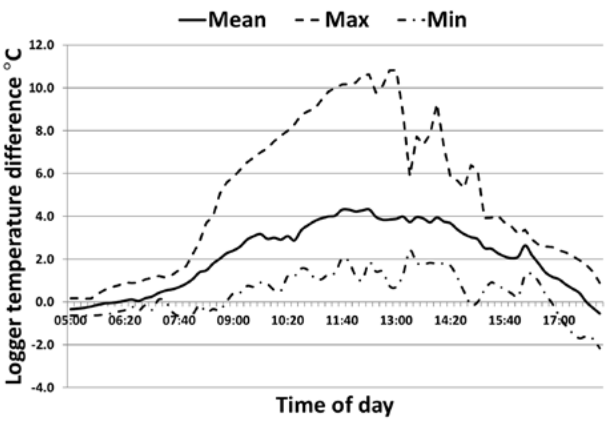

Figure 4. Mean and range of differences between temperature records of loggers under tins placed on mats or on the ground, recording at 10 minute intervals one day each month from March to October

thus forcing them to seek cooler locations. Inspection of the temperature records from the loggers shows this to be the case for periods of one to nearly five hours in five of the eight months (Table 1). However, only once was $T_{set\ upper}$ exceeded on the adjacent bare ground below the tin and this was for only 2 hours, yielding a very low degrees*minutes product (Table 1). Consequently, it would be expected that vipers frequently have the opportunity to move from the mat onto the bare ground below the tin to enable them to remain within their preferred temperature range.

Table 1. Number of minutes, temperature range, and degree*minutes, above viper $T_{set\ upper}$ (32.9°C) recorded over one day (05.00h to 18.00h) from March to October by temperature loggers placed beneath tin refuges either on an insulation mat or on bare ground.

Month	On mat			On bare ground		
	Mins above	Degree range above	Degrees* mins. above	Mins above	Degree range above	Degrees* mins. above
March	0	0	0	0	0	0
April	290	0.17 - 4.29	613	120	0.03 - 0.91	61.12
May	10	0.22	2	0	0	0
June	290	0.4 - 4.46	731	0	0	0
July	60	0.48 - 4.36	169	0	0	0
August	0	0	0	0	0	0
Sept	170	0.21 - 4.4	471	0	0	0
Oct	0	0	0	0	0	0

Observations of vipers under insulated and uninsulated tins

There were significantly more vipers (Mann Whitney $U = 3$, $n = 7$, $p = 0.009$) recorded at insulated than uninsulated tins (Table 2, a v. b). Vipers were observed at a total of 12 locations under insulated tins and 9 locations under uninsulated tins (i.e. 21 out of the 46 refuge locations were in use by vipers). When vipers were found under insulated tins, twice as many were resting on the insulation mat as on the bare ground (Table 2, c v. d), a statistically significant difference (Mann Whitney $U = 10.5$, $n = 7$, $p = 0.049$).

The majority of observations of adults and juveniles were at insulated tins while the sub-adults (comprising mostly males) were evenly divided between the two (Table

Table 2. Number of *V. berus* under insulated and uninsulated tins, and for insulated tins the numbers of observations of vipers on or off the insulation mats.

Month	No. viper records			
	Under insulated tins (a)	Under insulated tins (b)	Under insulated tins on mat (c)	off mat (d)
March	3	2	3	0
April	12	7	9	3
May	20	5	14	6
June	18	8	10	8
July	11	8	6	4
August	16	6	12	4
Sept	8	2	4	4
Totals	**88**	**38**	**58**	**29**

3). There was significant heterogeneity between the numerical values for the numbers of vipers using the mat or using the ground below insulated tins ($\chi^2 = 25.88$, df = 3, p<0.001). Significantly greater numbers of adults were observed on mats than immatures, while adults themselves differed with significantly more males than adult females, while the sub-adults and juveniles were not significantly different from each other.

Table 3. Numbers of records of vipers, by life stage, under insulated tins or uninsulated tins and numbers resting on the insulation mat (not including repeated observations on the same day). Values not followed by the same letter are statistically significant (χ^2 p<0.05).

	Adult males	Adult females	Sub-adults	Juveniles
Uninsulated tins	1	11	22	4
Insulated tins	21	31	22	16
% at insulated tins	95.5%	73.8%	50.0%	77.8%
- of which on mats	21	23	9	5
- of which on ground	0	8	13	11
% on insulation mats	100% a	74.2% b	40.9% c	35.7% c

The T_b of all viper life stages was on average warmer than T_e, whether under insulated or uninsulated tins (Table 4). There were apparent differences in viper T_b between insulated and uninsulated tins; adult males had an average 4°C advantage under insulated tins, adult females 1.8°C, juveniles 1°C but sub-adults were on average 0.7°C cooler (Table 4). The small number of observations under uninsulated tins precludes statistical comparisons with T_b under insulated tins. However, when taken together, the life stages under insulated tins did not show any statistical heterogeneity so further statistical testing was not justified (Kruskal Wallis, H = 1.4, df = 3, p = 0.7). In the case of the adult males the strong advantage under insulted tins resulted from eight high readings (9.4° to 15.8°C of difference) in late March early April. If these eight readings for males are removed from the estimate then the mean difference falls to +1.2°C±3.0°, comparable to adult males under uninsulated tins. The large differences in March and April are explained by the particularly low T_e at this time when only males were active, and when only insulated tins were selected. At low T_e the potential difference between T_e and $T_{set\ upper}$ is large. However, as the season progressed other life stages started to emerge and use tins but the prevailing T_e rose so narrowing the range from T_e to $T_{set\ upper}$, and reducing the size of any potential differences.

Table 4. Mean (±sd) and median temperature deviation °C of *V. berus* body temperature (T_b) from models on bare ground under tins (T_e), for uninsulated and uninsulated tins (n = number of temperature measurements including some repeat measures on the same day).

		Adult males	Adult females	Sub-adults[1]	Juven-iles
Uninsulated tins	Mean±sd	+1.4±1.6	+0.4±2.5	+2.2±3.1	+1.1±2.4
	Median	-	-0.4	+0.76	+0.5
	n	2	15	21	3
Insulated tins	Mean±sd	+5.4±6.7	+2.2±3.7	+1.5±3.3	+2.1±3.6
	Median	+3.6	+2.5	+0.92	+2.04
	n	24	15	26	16

[1] Sub-adult females - 2 under uninsulated tins, 4 under insulated tins

DISCUSSION

The potential for low ground temperatures to limit refuge use suggested that an insulating layer placed between the refuge and ground could offer a thermally more beneficial environment (Hodges and Seabrook, 2016b). More vipers were recorded at insulted tins, which had insulation mats covering half the area below them, than under uninsulated tins. Furthermore, under insulated tins greater numbers of vipers were observed resting on the insulation mat than on the adjacent bare ground. Adult vipers particularly favoured insulated tins, sub-adults were equally divided between insulated and uninsulated tins, while juveniles, although more frequent under insulated tins, were at insufficient numbers to draw firm conclusions. Presumably the higher temperatures offered by the insulation mat either attracted the vipers, especially the adults, to move under the insulated tins and/or resulted in them remaining under these tins for longer.

The adult males emerge from hibernation early in the year and observation in March/early April showed individuals with body temperature (T_b) 9.4°-15.8°C warmer than the operational temperature (T_e) demonstrated by models on bare ground. Later in the year when T_e was higher, adult males, females and juveniles were on average about 1 to 2°C warmer when under insulated tins but, surprisingly, sub-adults showed no temperature difference. These values provide some circumstantial evidence of the thermal benefit of insulation mats for males, females and juveniles. The absence of any observed benefits for sub-adults is perplexing but may be explained by the fact that under insulated tins they were less often seen on mats than the other stages (Table 4). We do not know if this should be interpreted as them using the mats less frequently or that they heat up more quickly and so spend less time on mats. Unfortunately, in 2015 there were insufficient multiple temperature measurements of the same viper on the same day to draw any conclusions about the heating rate of vipers on insulation mats. However, simple logic would suggest it should be at a higher rate than for those vipers resting on the ground.

It might have been predicted that the thermal advantages of the insulation mat would largely be restricted to times when ground temperatures were low and there was plenty of sunshine, i.e. during the early months of the year.

Surprisingly, the insulated tins actually contributed greater numbers of viper observations throughout the period March to September. With the exception of September, under insulated tins the numbers of vipers on mats were greater than on bare ground (Table 2). This suggests that the climatic conditions in 2015 favoured the use of the insulation mat for most of the year. Nevertheless, temperature loggers placed on insulation mats did exceed the viper $T_{set\ upper}$ quite frequently and for extended periods. However, bare ground adjacent to the mat beneath the tin rarely exceeded $T_{set\ upper}$ and so vipers may have moved onto cooler ground to remain within their preferred temperature range. This is supported by observations in 2014 when models on the ground under tins were never observed to exceed a T_e of 38˚C and only 8% exceeded an upper set point of 32.9˚C (Hodges & Seabrook, 2016b). However, it is possible that insulated refuges might be less useful in years with particularly hot summers or in exceptionally dull years, or months, when there would be little difference between resting on the ground or on a mat.

It seems likely that insulation mats enable vipers to achieve body temperatures closer to $T_{set\ upper}$ more quickly and for longer periods. This would optimise the rate of physiological processes and may also encourage vipers (especially adults) to remain under for longer periods, not least because the closer vipers are to $T_{set\ upper}$ the lower will be the incentive for them to leave the refuge to find a warmer position. This could help to reduce predation especially from birds. Both slow worms and common lizards were also frequently seen resting on the mats and it is possible they receive similar benefits. It seems unlikely that the use of insulation mats would find a place in short-term presence/absence refuge surveys, but for long-term surveys linked to conservation they may offer benefits for both recording and to the vipers themselves. Further investigations are in progress to include an insulation mat as a component of a refuge design that offers a wide range of thermal options so that vipers, and possibly other species, can gain maximum benefit from refuge use.

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